

Fig. S4

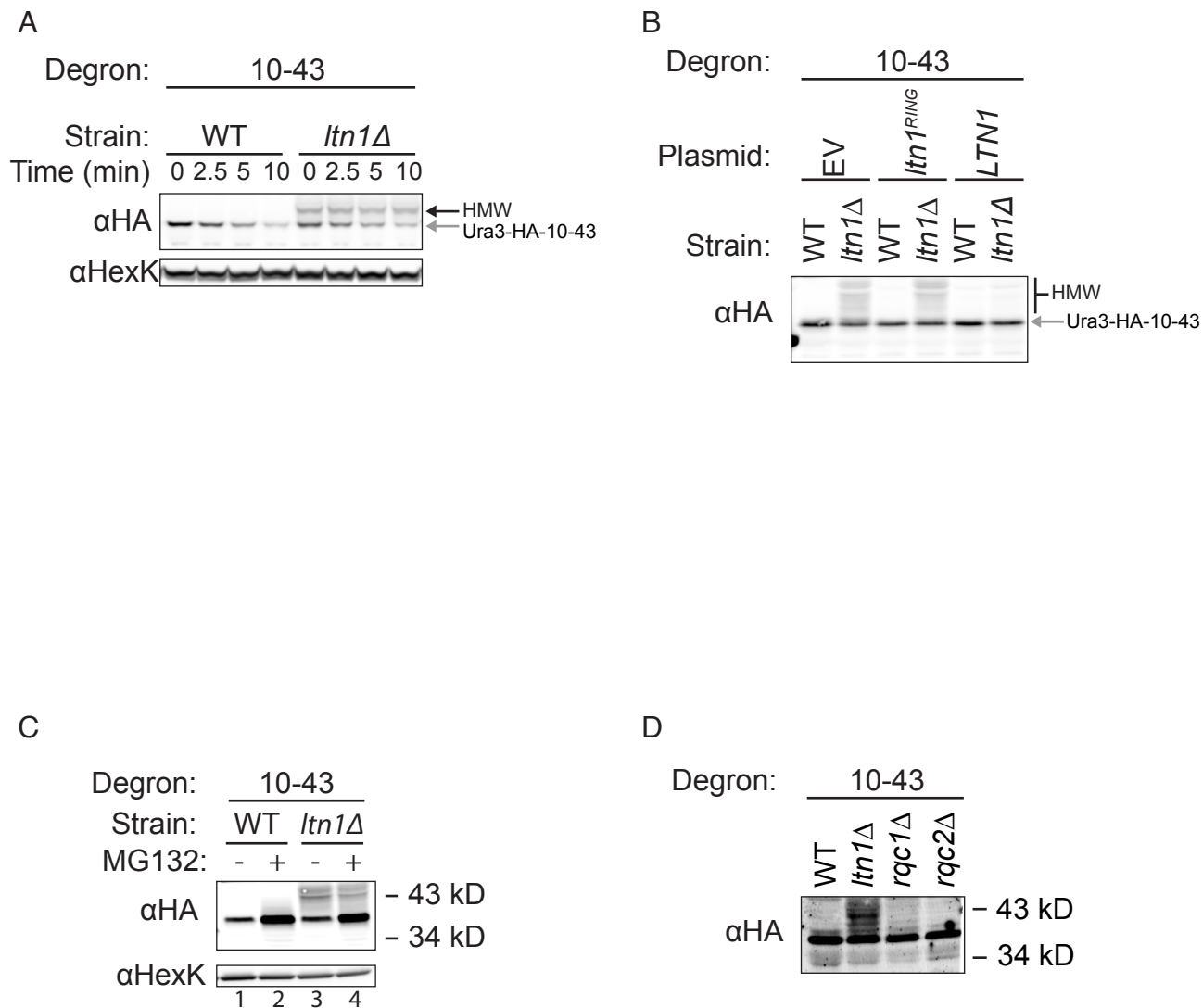


Figure S4. A proteasome-independent high MW species accumulates for the degron fusion protein Ura3p-HA-10-43 in the absence of the Ltn1p ubiquitin E3 ligase. (A) WT (SM4460) and *ltn1* Δ (SM5559) cells expressing Ura3-HA-10-43 (pSM2725) were analyzed by cycloheximide chase analysis as described in Materials and Methods. A high molecular weight (HMW) band or smear is observed only in the *ltn1* Δ strain. **(B)** *ltn1* Δ cells (SM5559) transformed with empty vector (pSM171), plasmid-borne *LTN1* (pSM2658) or *ltn1*^{RING} mutant (pSM2659) and Ura3-HA-10-43 (pSM2725) were analyzed by western blotting. **(C)** WT (SM4460) and *ltn1* Δ (SM5559) cells expressing Ura3-HA-10-43 (pSM2725) were treated with vehicle (DMSO) or drug (MG132; 75 μ M) for two hours prior to immunoblot analysis. **(D)** WT (SM4460), *ltn1* Δ (SM5559), *rqc1* Δ (SM6128) and *rqc2* Δ (SM6129) expressing Ura3-HA-10-43 were analyzed by western blotting.